

In the Specification

Please amend the specification as follows:

Please replace the paragraph on page 4, lines 1-8 with the following paragraph:

-- In a compositional sense, the invention provides a peptide that selectively binds to colon cancer cells, the peptide preferably is a cyclic peptide. The peptide has the formula: A-X1-X2-X3-X4-X5-X6-X7-X8-X9-B, wherein X1-X9 each are an amino acid, wherein A and B are absent or are amino acids or peptides containing up to 6 amino acids, and wherein amino acids X2, X3, X4, and X5 may be the same or different and each optionally may be absent. Preferably, the peptide is cyclic and is made up of the sequence cys-pro-ile-glu-asp-arg-pro-met-cys (SEQ ID NO: 1), where the peptide contains a disulfide bond between the cys side chains. --

Please replace the paragraphs on page 4, lines 22-24 with the following paragraphs:

-- Figure 4 shows the dependence of HT29 binding on the presence of three amino acids, RPM, and their position within the peptide. CPIEDRPMC, CPIRPMEDC, CPIDERPMC, CALRDRPMC, CPEKFRPMC AND CSPQSQPMC sequences are disclosed as SEQ ID NOS 134, 137 and 142-145, respectively.

Figure 5 shows binding data demonstrating that phage displaying peptides containing the RPM motif bind selectively and specifically to HT29 cells. CPIEDRPMC CKHLGPQLC, CPIEDRPMCGGS and CKHLGPQLCGGS sequences are disclosed as SEQ ID NOS 134-135 and 140-141, respectively. --

Please replace the paragraph on page 5, lines 8-9 with the following paragraph:

-- Figure 9 shows immunofluorescence data demonstrating that a peptide containing the RPM motif binds to colon tumors. CPIEDRPMC sequence is disclosed as SEQ ID NO: 134.--

Please replace the paragraph on page 5, lines 12-13 with the following paragraph:

--Figure 11 shows that a peptide containing the RPM motif conjugated to a cytotoxic agent can kill colon tumor cells. RPM-KLAK and KLAK sequences are disclosed as SEQ ID NOS 132-133, respectively. --

Please replace the paragraphs on page 10, line 2 to page 11, line 7 with the following paragraphs:

-- In another embodiment, the peptides contain a cyclic nonapeptide motif, where X1 and X9 are cys, X6-X8 are arg-pro-met, X2 is pro; X3 is ile or leu; X4 is glu, asp, or arg; and X5 is asp or glu. In another embodiment, the peptides contain a cyclic nonapeptide motif, where X1-X9 is cys-pro-ile-glu-asp-arg-pro-met-cys (SEQ ID NO: 1). In each of the embodiments where X1 and X9 are cys, the peptide is cyclized via a disulfide bond between the side chains of X1 and X9. In yet another embodiment, the peptide contains a arg-pro-met-cys (SEQ ID NO: 2) motif, which may be constrained in a cyclic structure, wherein the cys-side chain forms a disulfide bond with another cysteine side chain located elsewhere in the peptide.

In yet further embodiments, X1 and X9 are cys, and X2-X8 are selected, respectively, from the group consisting of (SEQ ID NOS 6-112, respectively, in order or appearance):

| | | | | | |
|---------|---------|---------|---------|---------|---------|
| ALLPNKT | AQPLKQN | SMSSHRW | APSQRAQ | AYPYWLY | SNSQDQN |
| ELNAAHT | DHPVPWR | SPQSQPM | ETGYSFR | DLREHTL | SRLDSPF |
| ETLSPRD | DRIGARQ | SYDYAKH | FESQSRL | FESQSRL | THLMPLT |
| FMKTLSN | GTATLHW | TKSLLLA | HQLYRGL | HDSLYRA | TSPLPSQ |
| IQGSGST | HNPPRPQ | TSSTPKA | KASMKSP | HNVRFPN | TTRGPST |
| KATAMNS | HQSSPQL | VSLQPMT | LAHASNS | HQTNPNE | VSNQIAN |
| LAKVPAS | HSSHTHQ | VTTLNLT | MLPHGRT | IDPSLGL | NFNSRAS |
| IHPVPWR | NGTSRIQ | KAESPME | NLKQPEH | KATMTAT | NRALHSY |
| KDKDNLP | NSARWSV | KLVPTHQ | NSHDPEN | KNERAYL | NSKDPGT |
| KNLTHKH | NVTWGDT | KPTLPLS | PATPLKF | KQHHVTE | PKGSGMN |
| KQPTSNY | PNQGAYV | KSPSSLQ | PPAHPN | KTPIPKI | QLPRSQS |
| KTTHPAL | QQSLSLI | LHMHQHI | QTPSLRL | LKQHWYS | SAHHPHA |
| LLPLAAP | SHQDPSL | LPHSQAQ | SLSQPFR | LPSKFSH | SSRPPWN |
| LSASTLM | THSHKKP | LSPISLQ | TNPMRLH | LTPEPQY | TQLPVSW |
| NASLMSV | TTWWAST | NATQWQH | VHKFKPF | NGSYVWR | NPNSNDM |

NSMPLHA NWQPATH PFGMVHT PHPWPGK PKMLGAA PLTPPTV
PPHTLGL PQELHPN PSNETTQ PSTAELA PSYSTSY PVSNLLQ
QPPMFYS QPQSQPM QTTPPFL QWAALRP and SLRTAAA.

Mixtures or pools of the peptides in any combination may be used, but the invention also contemplates the use of a single type of peptide.--

Please replace the paragraph on page 13, line 18 to page 14, line 4 with the following paragraph:

-- The drug or modified toxin is then treated by methods known to those skilled in the art to permit them to be conjugated to the protein containing at least one mercapto group. Methods for treating toxins and, in particular, modified *Pseudomonas* exotoxins, are disclosed in Batkra et al., Proc. Natl. Acad. Sci. USA, Vol. 86, pp. 8545-8549, 1989; Seetharam et al., The Journal of Biol. Chem., Vol. 266, No. 26, pp. 17376-17381, 1991; and Pastan et al., U.S. Pat. No. 4,892,827, all incorporated herein by reference. A preferred modified *Pseudomonas* exotoxin comprises ADP ribosylating activate, an ability to translocate across a cell membrane and devoid of a functional receptor binder region Ia of the native toxin. One such modified *Pseudomonas* exotoxin is devoid of amino acids 1-252 and 365-380 of native *Pseudomonas* exotoxin and contains a-KDEL (SEQ ID NO: 3) mutation instead of -REDLK (SEQ ID NO: 4) at the carboxyl terminus.--

Please replace the paragraph on page 15, lines 1-9 with the following paragraph:

-- Indeed, the present inventors have generated a reagent that can eliminate a tumor (including the remains of tumor not in contact with the vasculature) by binding selectively to cancer cells and, by being internalized, by carrying a toxin to the cells. In vitro, HT29 colon cancer cells were selectively killed by incubating them with RPM conjugated to a toxin. In contrast, HCT116 (non tumor) cells were not affected by treatment with RPM-(KLAK)₂ (SEQ ID NO: 5). This demonstrates an exquisite selectivity of RPM for HT29 cells, as well as the utility and promise of this approach for selectively destroying tumor cells while leaving normal cells intact or substantially unharmed. The peptide was shown to be internalized upon binding to the tumor cells.

Please replace the paragraph on page 18, lines 18-23 with the following paragraph:

-- RPM-KLAK (SEQ ID NO: 132) and KLAK (SEQ ID NO: 133) on HT29 and HCT116 cells. HT29 and HCT116 cells were incubated with increasing log concentrations of RPM-KLAK (SEQ ID NO: 132) or KLAK (SEQ ID NO: 133) for 72 hours at 37°C. After incubation, MTT was added to a final concentration of 2.5 ug/mL and incubated at 37°C for 1 hour. The media/MTT was removed carefully and the cells dissolved with 0.1N HCl in isopropanol. Absorbance was read on a plate reader (Cytofluor II, Perceptive Biosystems) at 570 nm. --

Please replace the paragraph on page 20, line 13 to page 21, line 9 with the following paragraph:

-- The enrichment of peptides with the RPM sequence suggested that binding to HT29 cells depended on the peptide displayed by the phage and not on an incidental property of the phage. To determine whether CPIEDRPMC (SEQ ID NO: 134) displaying phage bind to HT29 cells specifically, a competition assay was performed using phage and synthetic peptides, peptides corresponding to the specific sequence, CPIEDRPMC (SEQ ID NO: 134), and the non-specific sequence, CKHLGPQLC (SEQ ID NO: 135), were synthesized by standard methods and oxidized to form a disulfide bond. Reaction of the peptides before and after oxidation with Ellman's reagent was performed to verify presence of the disulfide bond. CKHLGPQLC (SEQ ID NO: 135) was isolated from a previous screen for peptides that bound to HT29 cells and as such bound to HT29 cells. In Figure 2A, HT29 cells were incubated with increasing log concentrations of CPIEDRPMC (SEQ ID NO: 134) peptide and with a constant number of either specific or non-specific phage. The number of phage binding to the HT29 cells was quantified and the percentage binding calculated based on the number of phage that bind in the absence of competitor. The sequence CPIEDRPMC (SEQ ID NO: 134) was competed with specific competitor but not with the non-specific competitor. Specific competitor reduced the level of RPM associated binding to HT29 cells to less than 4% whereas the level of binding to HT29 cells remained constant at 100% in the presence of non-specific competitor (Figure 2A). The reciprocal experiment was also done with HT29 cells incubated with increasing log concentrations of specific or non-specific peptide and a constant concentration of phage displaying the peptide CPIEDRPMC (SEQ ID NO: 134). The results are similar to what was observed in Figure 2A in that a peptide sequence corresponding to CPIEDRPMC (SEQ ID NO: 134) was able to compete phage bearing the same sequence but a peptide sequence

CKHLGPQLC (SEQ ID NO: 135) was not able to compete phage bearing the CPIEDRPMC (SEQ ID NO: 134) sequence. --

Please replace the paragraph on page 22, line 19 to page 23, line 7 with the following paragraph:

-- ***Binding to HT29 Cells is Dependent on the Amino Acids, RPMC-C (SEQ ID NO: 136).***

The ability of the peptide to bind selectively to human colon tumors was intriguing and prompted characterization of the interaction of the peptide with HT29 cells. The phage selection procedure produced naturally occurring substitution mutants so the phage and the peptide could be used to determine a functional consensus sequence for the family. HT29 cells were incubated with increasing log concentrations of CPIEDRPMC (SEQ ID NO: 134) peptide and a constant number of phage displaying one of the sequences were selected. The kinetics of competition were almost identical (within 2 fold) for the phage displaying the RPM motif. In contrast, the phage bearing the peptide sequence PM was competed at a 40 fold lower concentration of peptide than the phage bearing the RPM motif. The identity of the 4 residues before the RPM motif were unimportant and did not effect the ability of the peptide to compete with the phage for binding to HT29 cells. --

Please replace the paragraph on page 23, lines 17-22 with the following paragraph:

-- Since all of the peptides isolated from the selection contained the RPM motif at the C-terminal end of the peptide directly adjacent to the final cysteine, the importance of the cysteines for RPM binding activity was examined. A peptide was prepared bearing the RPM motif in the middle of the peptide (RPM middle), CPIRPMEDC (SEQ ID NO: 137), and used in the phage competition assays. Figure 5C shows that the (RPM) middle peptide was over 100 fold less potent when compared to RPM in competing with the phage for HT29 cell binding. --

Please replace the paragraph on page 25, line 6-19 with the following paragraph:

-- Since RPM-FITC is internalized at 37°C, RPM may be used to deliver a toxin to HT29 cells. To demonstrate this, an antimicrobial toxin, (KLAK)₂ (SEQ ID NO: 138), was used that selectively disrupts the mitochondrial membrane of eukaryotic cells while the cytoplasmic membrane remains intact. Cells that were incubated with (KLAK)₂ (SEQ ID NO: 138) alone or with free peptide were refractory to death induced by the toxin. However, when a peptide that

was specific and also internalized by cells was conjugated to (KLAK)₂ (SEQ ID NO: 138), the cells were killed. RPM was coupled to (KLAK)₂ (SEQ ID NO: 138) and incubated with HT29 and HCT116 cells with either (KLAK)₂ (SEQ ID NO: 138) alone as a negative control or RPM-(KLAK)₂ (SEQ ID NO: 5). Cells were treated for 72 hours at 37°C. At the end of the incubation, cell viability was measured by MTT assay. As expected, incubation of either cell line with (KLAK)₂ (SEQ ID NO: 138) did not effect the viability of the cells (Figure 7B). Since RPM should not bind to HCT116 cells, HCT116 cells should be resistant to death induced by RPM-(KLAK)₂ (SEQ ID NO: 5). This was indeed observed for HCT116 incubation with RPM (KLAK)₂ (SEQ ID NO: 5) (Figure 6B). In contrast, HT29 cell viability was affected by incubation with RPM-(KLAK)₂ (SEQ ID NO: 5) in a concentration dependent manner with an LC50 of 0.31 uM. --